

Bird Goën &amp; Co. PCT/BE/00120

1 T1639-PCT

8 October, 2001

**CLAIMS**

1. Method of positively identifying viable, committed, pluripotent skeletal precursor cells that have entered a post-natal differentiation pathway leading to skeletal or connective tissues comprising the steps of:  
isolating mammalian cells into a cell culture in vitro, and  
detecting the presence of a positive embryonic marker of an expressed morphogenic protein, a homolog thereof or a marker co-expressed and/or co-detectable with this marker.
2. The method according to claim 1, wherein the presence of the positive marker is further characterised by the absence of a negative marker.
3. The method according to claim 2, wherein the negative marker is FGFR3 or a marker or factor co-expressed or co-detectable with this negative marker.
4. The method according to any previous claim wherein the positive marker is an actively expressing gene, a protein or an mRNA expressed by a gene in the precursor cells or a part thereof, detectable at the DNA, mRNA, cDNA or the protein level and/or detectable via the activity of a promoter directing/regulating this gene expression, operably linked to a heterologous reporter gene.
5. The method according to any previous claim wherein the positive marker identifies precursor cells belonging to a joint interzone in mammals.
6. The method according to any previous claim wherein the expressed morphogenic protein is the cartilage-derived morphogenic protein CDMP-1 or a transforming growth factor  $\beta$  having at least 80% homology with CDMP-1 as a marker of skeletal precursor cells from any part of the body or a marker or factor co-expressed or co-detectable with any or all of these positive markers.
7. A method according to any previous claim, wherein  
the step of detecting the presence of the positive marker includes applying a

AMENDED SHEET

Empfangszeit 9. OKT. 11:01

09-10-2001

Bird Goen &amp; Co. PCT/BE/00120

2 T1639-PCT

8 October, 2001

binding agent for the positive marker to an isolated source of cells having the precursor cells, the marker positively identifying the viable skeletal precursor cells; and

separating the cells which are bound to the binding agent.

8. Use of reagents, ligands, and/or antibodies recognizing cell surface markers for sorting and enriching of precursor cells in cell culture in vitro, wherein the cell surface marker is co-expressed or co-detectable with the marker of any of claims 1 to 7.
9. Use of reagents, ligands, and/or antibodies recognizing cell surface markers according to claim 8, for sorting of skeletal precursor cells and for enriching a population in skeletal precursor cells.
10. Use for enriching via cell sorting according to claim 8 or 9, wherein cell sorting methods comprise fluorescence activated cell sorting, the use of magnetic beads coated with the respective antibodies or ligands, the use of affinity chromatography or the use of any other means coated with antibodies or ligands directed to the cells which are to be selected.
11. Use of reagents and/or antibodies according to any of claims 8 to 10 wherein the antibodies are polyclonal or monoclonal antibodies.
12. Use of skeletal precursor cells marked according to any of claims 1 to 7 for producing or repairing connective tissue in a mammal.
13. Use according to claim 12, wherein the said cells are cultured at a cell density of at least  $10^5$  cells/ml.
14. Use according to claim 12 or claim 13, comprising further administration of a factor that stimulates differentiation of the skeletal precursor cells into the type of connective tissue to be produced or repaired.
15. Use of precursor cells marked according to any of claims 1 to 7 as a source of

growth factors.

16. Use of precursor cells marked according to any of claims 1 to 7 as matrix producing cells.
17. Use according to claim 16, wherein the said matrix further comprises a bio-resorbable polymer or carrier.
18. Use according to claim 16 or 17 for the treatment of subglottic stenosis, tracheomalacia, chondromalacia patellae, osteoarthritis and traumatic lesions in a mammal.
19. A procedure for joint surface defect repair in a mammal comprising the co-implantation of skeletal precursor cells marked according to any of claims 1 to 7 and chondrocytes.
20. A method for enhancing the implantation of a prosthetic device in connective tissue comprising the step of implanting a prosthetic device having skeletal precursor cells according to any of the claims 1 to 7 adhered thereto under conditions suitable for differentiating the cells into the connective tissue desired.
21. A culture of isolated and expanded, viable, differentiated, pluripotent, precursor cells that have entered a post-natal differentiation pathway leading to skeletal or connective tissue, wherein the cells express a positive embryonic marker which is an expressed morphogenic protein, a homolog thereof or a marker co-expressed and/or co-detectable with this marker.
22. A therapeutic composition comprising the cells of claim 21.
23. An implant comprising the cells of claim 21.
24. The implant of claim 23 suitable for connective tissue implantation.
25. A method of treating a patient in need thereof comprising administration of the

therapeutic composition of claim 22.

26. A diagnostic for positively identifying in vitro a positive marker of viable, committed, pluripotent, skeletal precursor cells that have entered a post-natal differentiation pathway leading to skeletal or connective tissues, wherein the marker is an expressed morphogenic protein, a homolog thereof or a marker co-expressed and/or co-detectable with this marker.
27. The diagnostic according to claim 26 wherein the diagnostic also identifies the absence of a negative marker.
28. The diagnostic wherein the positive marker identifies precursor cells belonging to a joint interzone in mammals.
29. The diagnostic according to any of claims 26 to 28 wherein the expressed morphogenic protein is the cartilage-derived morphogenic protein CDMP-1 or a transforming growth factor  $\beta$  having at least 80% homology with CDMP-1 as a marker of skeletal precursor cells from any part of the body or a marker or factor co-expressed or co-detectable with any or all of these positive markers.
30. Use of an embryonic marker to positively identify viable, committed skeletal pluripotent precursor cells that have entered a post-natal differentiation pathway leading to connective or skeletal tissues, wherein the embryonic marker is an expressed morphogenic protein, a homolog thereof or a marker co-expressed and/or co-detectable with this marker.